# OSMOTIC STUDIES OF AMPHIBIAN EGGS. III. OVULATED EGGS

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In the two previous papers in this series (de Luque and Hunter, 1959; Hunter and de Luque, 1959) a study was made of the movement of water and cations into and out of the eggs of *Bufo marinus* and *Hyla labialis* with the emphasis on ovarian eggs. It was hoped that by making a similar study with fertilized and unfertilized eggs laid in water, it might be possible to find a basis to explain the apparent decrease in osmotic pressure of these eggs after they have left the ovary. These observations are included in the present paper.

## Materials and Methods

As in the previous experiments, *Hyla labialis* were obtained locally and *Bufo marinus* were shipped to Bogotá by air. *Bufo* pituitaries suspended in 0.66% NaCl were injected to obtain ovulation. Since with these two species, sperm suspensions, obtained by removing the testes from a male, cannot be used to fertilize the eggs, the males also had to be injected. It has now been established (A. S. Hunter, unpublished data) that one can find *Hyla* with mature ova twice a year, associated with the rainy seasons. Pituitary injections at these two seasons usually result in fertilized eggs. However, in the case of *Bufo* nothing is known about their normal season in Colombia for ovulation, and consequently fertilized eggs were obtained from this species only occasionally.

As previously described, the jelly layer was removed from the eggs with watch-maker's forceps and the eggs were suspended in various NaCl and KCl solutions. Since volume changes have previously been reported, only a few of these measurements were made. Water and Na and K content were measured as before. A new attempt was made to measure non-solvent water using a distribution technique (*cf.* Parpart and Shull, 1935). The quantity of glycerol was determined by the method of Bailey (1959).

## Results

*Bufo ovarian eggs.* In the first paper of this series it was suggested that *Bufo* ovarian eggs separated from their follicular layers swelled less than these eggs plus their follicular layers. In those experiments volume changes of eggs plus layers were measured after a short exposure and after a longer exposure to solutions with different osmotic pressures. Similar measurements using eggs without layers were made only after short exposures to the solutions since such eggs rapidly become aspherical. Since the apparent difference in volume changes

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between eggs with and without their follicular layers could have resulted simply from a difference in time of exposure, these experiments were repeated with observations at the end of an hour and at the end of 24 hours. At the end of an hour's exposure to solutions of NaCl (0.75–0.55%) the same small volume changes were observed with both types of eggs. This is similar to the changes previously reported (Fig. 2, p. 462 and the open symbols in Fig. 1, p. 461). At the end of 24 hours, the eggs without follicular layers swelled appreciably in all of the solutions (*cf.* closed symbols in Fig. 1, p. 461) with larger volume changes occurring in more hypotonic solutions. Measurements of Na and K content of these eggs after 24 hours in these solutions also indicated that Na and K could move across the cell membrane. It can be concluded, then, that water and salts can move slowly into ovarian eggs and also into the cells forming their follicular layers.

TABLE I

*The amount of Na or K present in jelly following suspension in solutions of NaCl or KCl*

| Number of experiments | Na—meq./mm.$^3$ $\times 10^5$ | | | | | |
|---|---|---|---|---|---|---|
| | 0.60% NaCl | | 0.38% NaCl | | 0.20% NaCl | |
| | Observed | Calculated | Observed | Calculated | Observed | Calculated |
| 8 | 9.9 | 10.2 | 8.0 | 6.5 | 5.6 | 3.4 |
| | K—meq./mm.$^3$ $\times 10^5$ | | | | | |
| | 0.60% KCl | | 0.38% KCl | | 0.20% KCl | |
| 7 | 7.6 | 8.0 | 5.1 | 5.1 | 2.9 | 2.7 |

*Ovulated eggs.* In all of the experiments made using eggs laid in water, whether fertilized or not, interpretation of the data becomes difficult because of the presence of a layer of jelly of varying thickness surrounding the eggs. The majority of the jelly was removed but it is almost impossible to obtain a completely clean egg on which to experiment. Moreover, if all of the jelly is removed the eggs do not develop normally (a higher percentage of exogastrulae results). For this reason, studies had to be made of isolated jelly in order to determine its contribution to the results obtained from eggs with a small jelly layer.

Since it was difficult to obtain sexually active males and females of *Bufo* simultaneously, only a few samples of *Bufo* fertilized eggs were studied. Because of the theoretical importance of a possible change in the amount of non-solvent water in the ovulated eggs (as compared to ovarian eggs) all of the samples of fertilized *Bufo* eggs were used for these studies, which unfortunately did not give repeatable results. The data on water, Na and K content of isolated jelly and of laid (unfertilized) *Bufo* eggs and of fertilized *Hyla* eggs follow.

*Jelly.* The jelly will be considered first, since it is involved in all of the other determinations. The difference between wet and dry weight showed that at least

TABLE II

*Changes in water content of unfertilized eggs of Bufo marinus placed in solutions of NaCl of different osmotic pressures*

| Bufo no. | 0.60% NaCl | | 0.38% NaCl | | 0.20% NaCl | | Water | |
|---|---|---|---|---|---|---|---|---|
| | % | mg./egg | % | mg./egg | % | mg./egg | % | mg./egg |
| 1 | 87 | 4.96 | 90 | 6.02 | 87 | 4.97 | 95 | 13.39 |
| 2 | 88 | 6.16 | 86 | 5.09 | 89 | 6.69 | | |
| 3 | 95 | 5.37 | 96 | 9.50 | 95 | 6.47 | | |
| Av. | 90 | 5.50 | 91 | 6.87 | 90 | 6.04 | | |

TABLE III

*Changes in salt content (meq./mg. $\times 10^5$) of unfertilized eggs of Bufo marinus placed in solutions of NaCl of different osmotic pressures*

| Bufo no. | 0.60% NaCl | | 0.38% NaCl | | 0.20% NaCl | | Water | |
|---|---|---|---|---|---|---|---|---|
| | Na | K | Na | K | Na | K | Na | K |
| 1 | 24.8 | 14.0 | 21.8 | 14.8 | 19.0 | 14.0 | 7.3 | 15.0 |
| 2 | 33.4 | 16.0 | 27.6 | 16.4 | 18.6 | 14.0 | | |
| 3 | 25.8 | 4.8 | 27.7 | 4.5 | 13.0 | 3.9 | | |
| Av. | 28.0 | 11.6 | 25.7 | 11.9 | 16.9 | 10.6 | | |

TABLE IV

*Changes in water content of unfertilized eggs of Bufo marinus placed in solutions of KCl of different osmotic pressures*

| Bufo no. | 0.60% KCl | | 0.38% KCl | | 0.20% KCl | |
|---|---|---|---|---|---|---|
| | % | mg./egg | % | mg./egg | % | mg./egg |
| 1 | 89 | 5.42 | 88 | 5.96 | 90 | 6.91 |
| 2 | 82 | 4.44 | 88 | 6.68 | 87 | 6.24 |
| 3 | 87 | 3.09 | 88 | 3.77 | 93 | 6.42 |
| Av. | 86 | 4.32 | 88 | 5.47 | 70 | 6.52 |

TABLE V

*Changes in salt content (meq./mg. $\times 10^5$) of unfertilized eggs of Bufo marinus placed in solutions of KCl of different osmotic pressures*

| Bufo no. | 0.60% KCl | | 0.38% KCl | | 0.20% KCl | |
|---|---|---|---|---|---|---|
| | Na | K | Na | K | Na | K |
| 1 | 13.5 | 45.0 | 6.8 | 29.2 | 7.7 | 39.2 |
| 2 | 7.4 | 35.5 | 8.2 | 23.2 | 5.8 | 20.2 |
| 3 | 8.0 | 59.9 | 3.1 | 23.3 | 4.3 | 28.7 |
| Av. | 9.6 | 46.8 | 6.0 | 25.2 | 5.9 | 29.4 |

TABLE VI

*Changes in water content of fertilized eggs of Hyla labialis placed in solutions of NaCl of different osmotic pressures*

| Hyla no. | 0.60% NaCl | | 0.50% NaCl | | 0.38% NaCl | | 0.20% NaCl | | Water | |
|---|---|---|---|---|---|---|---|---|---|---|
| | % | mg./egg | % | mg/.egg | % | mg./egg | % | mg./egg | % | mg./egg |
| 1 | 75 | 1.93 | 78 | 2.01 | 79 | 2.30 | 81 | 3.11 | 87 | 4.24 |
| 2 | 65 | 1.43 | 77 | 1.82 | 72 | 1.45 | 75 | 1.60 | 90 | 5.93 |
| 3 | 80 | 1.92 | | | 82 | 2.96 | 83 | 2.40 | | |
| 4 | 71 | 1.52 | | | | | 82 | 3.06 | | |
| Av. | 76 | 1.70 | 78 | 1.92 | 78 | 2.24 | 80 | 2.54 | 89 | 5.09 |

99.5% of the jelly of both types of eggs is water. Solvent water measurements, which in the case of jelly are probably valid, showed that 100% of the water in the jelly is solvent. In 6 determinations on *Hyla* jelly, values between 98 and 144% were obtained.

*Hyla* jelly was placed in NaCl and KCl solutions of different concentrations. After one hour the Na and K content of the jelly was determined. In Table I a comparison is made between the amount of Na or K present in the jelly after one hour in the various solutions and the theoretical amount expected with diffusion equilibrium. In solutions of NaCl, no K was present in the jelly and vice versa. From this it can be concluded that normally there is little, if any, Na or K in jelly.

It can readily be seen that in all of the six solutions there is good agreement between the calculated and observed values. It can be concluded that in solutions of NaCl and KCl the jelly reaches diffusion equilibrium rather rapidly.

To calculate the volume of jelly surrounding each egg, measurements were made in water of the diameter of the egg and of the egg plus jelly. In one series of experiments the average volume of an egg was found to be 1.98 mm.$^3$ and the volume of the egg plus jelly, 5.60 mm.$^3$. Even when extreme care was used to remove as much jelly as possible without injuring the eggs these volumes were 1.65 mm.$^3$ for an egg and 2.86 mm.$^3$ for egg plus jelly. Similar measurements of eggs in 0.38% NaCl gave an average value of 1.03 mm.$^3$ for the volume of an egg and 2.11 mm.$^3$ for the volume of egg plus jelly. The difference between these

TABLE VII

*Changes in salt content (meq./mg. $\times$ $10^5$) of fertilized eggs of Hyla labialis placed in solutions of NaCl of different osmotic pressures*

| Hyla no. | 0.60% NaCl | | 0.50% NaCl | | 0.38% NaCl | | 0.20% NaCl | | Water | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Na | K | Na | K | Na | K | Na | K | Na | K |
| 1 | 15.8 | 18.0 | 10.6 | 13.1 | 8.1 | 13.2 | 10.0 | 13.5 | 4.1 | 11.9 |
| 2 | 17.2 | 14.0 | 10.8 | 11.2 | 11.4 | 11.8 | 4.5 | 8.5 | 4.8 | 12.9 |
| 3 | 19.6 | 10.5 | | | 16.2 | 14.0 | 11.7 | 8.7 | | |
| 4 | 13.7 | 10.1 | | | | | 16.0 | 14.7 | | |
| Av. | 16.6 | 13.2 | 10.7 | 12.2 | 11.9 | 13.0 | 10.7 | 11.4 | 4.5 | 12.4 |

TABLE VIII

*Changes in water content of fertilized eggs of Hyla labialis placed in solutions of KCl of different osomotic pressures*

| Hyla no. | 0.60% KCl | | 0.38% KCl | | 0.20% KCl | | Water | |
|---|---|---|---|---|---|---|---|---|
| | % | mg./egg | % | mg./egg | % | mg./egg | % | mg./egg |
| 1 | 81 | 2.42 | 84 | 2.93 | 80 | 2.53 | 83 | 2.86 |
| 2 | 61 | 1.73 | 72 | 1.91 | 63 | 1.45 | 84 | 4.08 |
| 3 | 75 | 2.17 | 77 | 2.46 | 73 | 2.20 | 88 | 5.64 |
| 4 | 64 | 1.26 | 67 | 1.44 | 72 | 2.05 | 85 | 4.09 |
| 5 | 72 | 2.05 | 77 | 2.83 | 60 | 1.22 | 83 | 4.60 |
| Av. | 71 | 1.93 | 75 | 2.31 | 70 | 1.89 | 85 | 4.25 |

values obtained in water and in 0.38% NaCl suggests that in water both the egg and the jelly swell but much more water is taken up by the jelly. It can be seen that in all of the subsequent experiments to be reported the volume of the jelly is larger than the volume of the eggs.

*Bufo, unfertilized eggs.* Tables II–V show the changes in water and salt content of unfertilized *Bufo* eggs after having been in various NaCl and KCl solutions during an hour. Since these eggs are always very irregular in form, volume measurements were not made.

*Hyla, fertilized eggs.* Similar data for *Hyla* fertilized eggs are presented in Tables VI–IX.

## ANALYSIS OF RESULTS

Because of the nature of the above experiments, various assumptions have to be made in trying to interpret the data. It is quite possible to change one's assumption and arrive at a completely different conclusion. The analysis which follows, then, represents one possible interpretation of the data. The only point the authors want to make is that the data are not inconsistent with these suggestions.

In general, a comparison of the above data with those previously reported shows that all of the changes are less with ovarian eggs. From the data in Table X it can be seen that the volume of unfertilized eggs (plus jelly) of *Bufo* is much

TABLE IX

*Changes in salt content (meq./mg. $\times$ $10^5$) of fertilized eggs of Hyla labialis placed in solutions of KCl of different osmotic pressures*

| Hyla no. | 0.60% KCl | | 0.38% KCl | | 0.20% KCl | | Water | |
|---|---|---|---|---|---|---|---|---|
| | Na | K | Na | K | Na | K | Na | K |
| 1 | 7.9 | 28.6 | 8.8 | 18.9 | 7.5 | 15.5 | 7.8 | 15.0 |
| 2 | 5.9 | 15.8 | 3.7 | 10.5 | 5.1 | 28.0 | 4.6 | 7.2 |
| 3 | 5.6 | 14.2 | 5.5 | 12.8 | 2.9 | 25.2 | 5.5 | 8.0 |
| 4 | 6.0 | 12.8 | 6.0 | 12.6 | 4.7 | 25.1 | 5.1 | 8.4 |
| 5 | 5.0 | 13.8 | 4.7 | 13.1 | 3.2 | 25.6 | 4.7 | 8.2 |
| Av. | 6.1 | 17.0 | 5.7 | 13.6 | 4.7 | 23.9 | 5.5 | 9.4 |

larger than that of ovarian eggs. The unfertilized eggs also have more Na and K. Fertilized *Hyla* eggs (plus jelly) are much larger than ovarian eggs and in NaCl solutions they have more Na and K. The presence of the jelly obviously could contribute to this difference.

One interesting point of comparison is that in spite of the differences in quantity and percentage of water in the eggs in the different solutions (Tables II, IV, VI, and VIII) the dry weight per egg, as calculated from these data, is always essentially the same, as would be expected. The dry weight of a *Bufo* ovarian egg as previously published was 0.59 mg. The unfertilized eggs in NaCl and KCl solutions give values of 0.61–0.75 mg./egg. The published figure for *Hyla* ovarian eggs is 0.65 mg./egg. In the present experiments with fertilized eggs the values are 0.54–0.81 mg./egg. Considering the difference in size of eggs from different females it seems reasonable to say that the eggs neither gain nor lose considerable quantities of substances included in the dry weight when they leave the ovary.

It is obvious from the measurements mentioned earlier of the volume of an egg and the volume of its jelly that a large part of the volume changes in Tables II,

TABLE X

*A comparison of water (mg./egg) and salt content (meq./mg. × $10^5$) of ovarian and laid eggs in isotonic solutions (0.66% and 0.38%, respectively)*

| Type of egg | NaCl | | | KCl | | |
|---|---|---|---|---|---|---|
| | Water | Na | K | Water | Na | K |
| *Bufo*, ovarian | 1.17 | 12.2 | 7.8 | 0.93 | 2.6 | 12.9 |
| *Bufo*, non-fertilized | 6.87 | 25.7 | 11.9 | 5.47 | 6.0 | 25.5 |
| *Hyla*, ovarian | 0.64 | 7.2 | 3.1 | 1.47 | 5.8 | 15.7 |
| *Hyla*, fertilized | 2.24 | 11.9 | 13.0 | 2.31 | 5.7 | 13.7 |

IV, VI, and VIII may be due to changes in the amount of water in the jelly. To try to determine whether or not water enters or leaves the egg let us consider that 0.38% salt solution has one unit of osmotic pressure and that the volume of the water in the egg plus jelly is 1. The expected volume of water in any other solution can be calculated using the formula PV = constant. Table XI compares these calculated values with the actual changes in the volumes of the eggs.

With the exception of the first value, the changes in the volume of water are always less than would be expected. That is, in hypertonic solutions the eggs shrank less and in hypotonic solutions they swelled less than the calculated values. An average of the per cent deviation of the last 6 values gives a figure of 28%. Using the figures previously given (egg plus jelly, 2.11 mm.$^3$; egg, 1.03 mm.$^3$) 28% of 2.11 mm.$^3$ gave a value very similar to the quantity of water in an egg with a volume of 1.03 mm.$^3$. Using the other measurements (egg plus jelly, 5.60 mm.$^3$; egg, 1.98 mm.$^3$) a similar calculation leads to the same conclusion. This would suggest that at least the major portion of the change in water content occurred in the jelly. Tentatively, then, let us suggest that these studies of changes in water content might be interpreted as indicating a decreased rate of movement of water across the membrane of an egg after it has left the ovary.

TABLE XI

*A comparison of the theoretical and observed volume changes in water content*

| Egg | Solution | Water content | | |
|---|---|---|---|---|
| | | Calculated volume | Observed volume | % difference |
| *Bufo* | 0.60% NaCl | 0.63 | 0.62 | 2 |
| | 0.60% KCl | 0.63 | 0.79 | 25 |
| | 0.20% KCl | 1.90 | 1.20 | 37 |
| *Hyla* | 0.60% NaCl | 0.63 | 0.76 | 21 |
| | 0.50% NaCl | 0.76 | 0.85 | 12 |
| | 0.20% NaCl | 1.90 | 1.13 | 41 |
| | 0.60% KCl | 0.63 | 0.84 | 33 |

In Table XII an attempt was made to determine how much of the increase in salt in the egg could be attributed to salt dissolved in the jelly. Two methods were used to calculate the *Hyla* data. The volume of the egg was considered to be 1.03 mm.$^3$ (obtained from the measurements previously given) or 1.44 mm.$^3$ (obtained by adding the dry weight to the amount of water in an ovarian egg). Only the latter method (volume = 1.23 mm.$^3$) was used in calculating the *Bufo* data. It was assumed that this volume did not change in the different solutions. (Probably this assumption is not completely correct but the error introduced would not change the argument.) This value was subtracted from the total volume of water actually measured in the various solutions (Tables II, IV, VI, and VIII) to give a minimum value for the amount of water in the jelly. The volume of jelly water was then multiplied by the calculated milliequivalents of Na or K in the different solutions to give a theoretical value for the amount of Na or K in the water in the jelly, assuming diffusion equilibrium. These figures can then be compared with the total amount of Na or K per egg calculated on the basis of 0.65 mg. per egg as a dry weight of *Hyla* eggs and 0.59 mg. for *Bufo* eggs.

In the case of *Bufo* eggs, comparing the last two columns, with the exception of 0.20% KCl (these data are inconsistent in every respect), the increase in measured amounts of Na and K could have been due completely to the solution

TABLE XII

*A comparison between calculated values of Na and K in the jelly layer and observed values in eggs plus jelly*

| Solution | Ion | Calculated quantity in *Hyla* jelly | | Obs. *Hyla* | Calculated quantity in *Bufo* jelly (1.23 mm.$^3$) | Obs. *Bufo* |
|---|---|---|---|---|---|---|
| | | (1.03 mm.$^3$) | (1.44 mm.$^3$) | | | |
| 0.60% NaCl | Na | 6.9 | 2.7 | 10.8 | 44.0 | 16.5 |
| 0.38% NaCl | Na | 7.9 | 5.2 | 7.7 | 36.7 | 15.2 |
| 0.20% NaCl | Na | 5.1 | 3.7 | 7.0 | 16.3 | 10.0 |
| 0.60% KCl | K | 7.2 | 3.9 | 11.0 | 24.8 | 27.6 |
| 0.38% KCl | K | 6.5 | 4.2 | 8.8 | 21.6 | 14.9 |
| 0.20% KCl | K | 2.3 | 1.2 | 15.5 | 14.2 | 17.3 |

of these ions in the jelly. In the case of *Hyla* eggs, the same conclusion could be reached. This suggestion could also explain the observation that in the present experiments there is more salt in the eggs plus jelly in more concentrated salt solutions, while with the ovarian eggs the amount of salt in the eggs was essentially the same regardless of the concentration of the surrounding solution.

Another method to analyze the data to test the same point follows. If we assume that the concentration of Na or K in the jelly is the same as in the surrounding solution, and if we further assume that the amount of Na or K in the egg is constant, three simultaneous equations can be solved for each series of experiments to determine the amount of Na or K in the eggs. If we let $a$ = volume of jelly in mm.$^3$ and $b$ = milliequivalents of Na (or K) in the egg, then for *Bufo* eggs in NaCl solutions:

$$10.3 \times 10^{-5}a + b = 19.1 \times 10^{-5}$$
$$6.4 \times 10^{-5}a + b = 17.5 \times 10^{-5}$$
$$3.4 \times 10^{-5}a + b = 11.5 \times 10^{-5}$$

With these three equations an average value of 1.17 mm.$^3$ for the volume of the jelly was obtained. Using this value, the amounts of Na in the *Bufo* eggs in the three NaCl solutions were 7.0, 10.1 and $7.5 \times 10^{-5}$ meq./mg. These values are quite similar to those previously obtained with ovarian eggs. Making similar calculations for *Bufo* eggs in KCl solutions and for *Hyla* eggs in NaCl and in KCl solutions, the values for Na or K in the eggs in these experiments are similar to, or less than, the amount previously reported in ovarian eggs.

The above calculations would suggest that salts as well as water enter and leave ovarian eggs more rapidly than they enter and leave eggs that have left the ovary.

Another calculation which is of interest is that previously made with ovarian eggs (Hunter and de Luque, last line, p. 476). From the data in Table II, the dry weights of the eggs in each of the three solutions of NaCl can be calculated. Multiplying these dry weights by the values of Na and K in Table III, the total milliequivalents of cations per cell can be calculated. Dividing this by the amount of water per cell, the concentration (in milliequivalents of Na plus K per liter) can be calculated. Values of 44, 37 and 31 were obtained with these data. These values are to be compared with 140 previously calculated for ovarian eggs. Similar calculations with *Bufo* eggs in KCl solutions and *Hyla* eggs in NaCl and KCl solutions yielded values considerably lower than for ovarian eggs. Although it is difficult to assess the effect of the jelly in these calculations, one might conclude that the osmotic pressure of eggs that have left the ovary is less than the osmotic pressure of ovarian eggs.

## Discussion

The various measurements of volume changes of ovarian eggs, unfertilized and fertilized eggs in solutions with different osmotic pressures have shown that water can move into or out of the three different types of eggs. The studies of Na and K content also suggest that these cations can move across the membranes of the three types of eggs. One analysis of the present data leads us to the conclusion that a decrease in permeability to water and salts in eggs that have left the ovaries is not inconsistent with the data. As was mentioned in the review of the literature

in the first paper of this series, similar suggestions have previously been made, based on studies of various eggs.

If the above suggestion is correct, it could explain how an egg laid in water could survive without losing all of its salts and without swelling indefinitely due to the entrance of water. However, it does not explain the apparent decrease in osmotic pressure in the eggs when they are laid.

The present data do not support the idea that the decrease in osmotic pressure of the eggs after they have left the ovary results from the loss of salts or from the entrance of a large quantity of water. On the contrary, the data in Table XIII suggest that there is an increase in the amount of K in the eggs that have left the ovary. The values of $t$ (calculated by the Method of Fischer) indicate that there is no difference in the amount of Na in *Hyla* ovarian and fertilized eggs but there is a difference in K in these eggs significant at the 2% level. There is no significant difference in the amount of Na in *Bufo* ovarian and unfertilized eggs but a highly significant difference in the amount of K in these eggs.

In making the salt analyses of the egg homogenates, the proteins are precipitated with trichloracetic acid. This means that whatever quantities of Na and/or

TABLE XIII

*A comparison of the Na and K content of ovarian and non-ovarian eggs of Hyla labialis and of Bufo marinus*

| | meq./mg. $\times 10^5$ | | $t$ |
|---|---|---|---|
| | Ovarian | Ovulated | |
| *Hyla*, Na content | 4.4 ± 0.20 | 5.5 ± 1.22 | 0.32 |
| *Hyla*, K content | 5.4 ± 0.03 | 12.4 ± 0.95 | 4.10 |
| *Bufo*, Na content | 6.8 ± 0.32 | 7.2 ± 0.29 | 3.12 |
| *Bufo*, K content | 8.3 ± 0.25 | 12.3 ± 0.31 | 72.0 |

K are bound to the proteins of the egg are not included in the Na and K measurements. If one postulates: (1) that in the ovarian eggs some K is bound to proteins, and (2) that on leaving the ovary there is a change in the proteins which decreases their capacity to bind K, the observed increase in K could be explained. If this is true, one might also postulate that the amount of water bound to proteins decreases. Unfortunately, with the methods available in this laboratory, satisfactory values of non-solvent water could not be obtained. Using the glycerol-distribution technique with fertilized eggs of *Hyla*, the amount of glycerol that disappeared from the solution increased more or less indefinitely with time. The possibility that these eggs are metabolizing glycerol is being investigated. Because of the importance of determining whether or not there is a change in solvent water when the eggs leave the ovary, one of the authors (O. de L.) will continue these studies in another laboratory using isotopic techniques.

## SUMMARY

1. Studies of eggs of *Bufo marinus* and of *Hyla labialis* that have left the ovary indicate that there is no large change in the total amount of water nor of Na and K.

2. A possible interpretation of the data is that after the eggs have left the ovary there is a decrease in their permeability to water and to Na and K.

3. A statistically significantly higher quantity of K in these eggs might suggest a change in the binding-capacity of the egg proteins.

4. If such a change does occur, there might be a decrease in the amount of water bound to the proteins which might explain the lower osmotic pressure of eggs that have left the ovary. Attempts to obtain experimental data to support this hypothesis failed for technical reasons.

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